AUG 1 1 2005

SEQUENCE LISTING

110> ITOH, NOBUYA WAKITA, RYUHEI

<120> PROCESS FOR PRODUCING 3-HYDROXYCYCLOHEXANONE

<130> Q76481

<140> 10/617,034

<141> 2003-07-11

<150> JP 2002-205207

<151> 2002-07-15

<160> 15

<170> PatentIn Ver. 3.2

<210> 1

<211> 348

<212> PRT

<213> Corynebacterium pseudodiphtheriticum

<400> 1

Met Lys Ala Ile Gln Tyr Thr Arg Ile Gly Ala Glu Pro Glu Leu Thr 1 5 10 15

Glu Ile Pro Lys Pro Glu Pro Gly Pro Gly Glu Val Leu Leu Glu Val 20 25 30

Thr Ala Ala Gly Val Cys His Ser Asp Asp Phe Ile Met Ser Leu Pro 35 40 45

Glu Glu Gln Tyr Thr Tyr Gly Leu Pro Leu Thr Leu Gly His Glu Gly
50 55 60

Ala Gly Lys Val Ala Ala Val Gly Glu Gly Val Glu Gly Leu Asp Ile 65 70 75 80

Gly Thr Asn Val Val Val Tyr Gly Pro Trp Gly Cys Gly Asn Cys Trp 85 90 95

His Cys Ser Gln Gly Leu Glu Asn Tyr Cys Ser Arg Ala Gln Glu Leu 100 105 110

Gly Ile Asn Pro Pro Gly Leu Gly Ala Pro Gly Ala Leu Ala Glu Phe 115 120 125

Met Ile Val Asp Ser Pro Arg His Leu Val Pro Ile Gly Asp Leu Asp 130 135 140

Ala Ile Lys Arg Ser Leu Pro Lys Leu Arg Gly Gly Ser Tyr Ala Val 165 170 175 Val Ile Gly Thr Gly Gly Leu Gly His Val Ala Ile Gln Leu Leu Arg 180 185 His Leu Ser Ala Ala Thr Val Ile Ala Leu Asp Val Ser Ala Asp Lys 200 Leu Glu Leu Ala Thr Lys Val Gly Ala His Glu Val Val Leu Ser Asp 210 Lys Asp Ala Ala Glu Asn Val Arg Lys Ile Thr Gly Ser Gln Gly Ala Ala Leu Val Leu Asp Phe Val Gly Tyr Gln Pro Thr Ile Asp Thr Ala Met Ala Val Ala Gly Val Gly Ser Asp Val Thr Ile Val Gly Ile Gly 265 Asp Gly Gln Ala His Ala Lys Val Gly Phe Phe Gln Ser Pro Tyr Glu 280 Ala Ser Val Thr Val Pro Tyr Trp Gly Ala Arg Asn Glu Leu Ile Glu Leu Ile Asp Leu Ala His Ala Gly Ile Phe Asp Ile Ser Val Glu Thr Phe Ser Leu Asp Asn Gly Ala Glu Ala Tyr Arg Arg Leu Ala Ala Gly 330 Thr Leu Ser Gly Arg Ala Val Val Pro Gly Leu 340 <210> 2 <211> 1047 <213> Corynebacterium pseudodiphtheriticum <220> <221> CDS <222> (1)..(1047) <400> 2 atg aag gcg atc cag tac acg aga atc ggc gcg gaa ccc gaa ctc acg 48 Met Lys Ala Ile Gln Tyr Thr Arg Ile Gly Ala Glu Pro Glu Leu Thr 10 gag att ccc aaa ccc gag ccc ggt cca ggt gaa gtg ctc ctg gaa gtc 96 Glu Ile Pro Lys Pro Glu Pro Gly Pro Gly Glu Val Leu Leu Glu Val

acc gct gct ggc gtc tgc cac tcg gac gac ttc atc atg agc ctg ccc Thr Ala Ala Gly Val Cys His Ser Asp Asp Phe Ile Met Ser Leu Pro 35

| | | | | | | | | 3 | | | | | | | |
|--------------|-------------------|---|---|---|---|---|-------|---|---|---|---|---|---|---|-----|
| gaa . Glu | gag Glu 50 | | | | | | | | | | | | | | 192 |
| _ | ggc Gly | _ | _ | - | _ | _ | | | _ | _ | | | _ | | 240 |
| | acc Thr | | _ | - | _ | | | | | - | | | _ | | 288 |
| | tgc Cys | | | | | | | _ | | _ | - | | _ | | 336 |
| | atc Ilc | | | | | | _ | | | _ | _ | _ | | | 384 |
| _ | atc Ile 130 | - | _ | | | _ | | - | _ | | | - | | _ | 432 |
| | gtc Val | | | | | | | | | | | | | | 480 |
| | atc Ile | | | | - | | | _ | | | _ | | _ | - | 528 |
| _ | att Ile | | | | | | | _ | _ | | _ | | | _ | 576 |
| | ctc Leu | _ | _ | - | _ | _ | _ | _ | - | _ | _ | | _ | _ | 624 |
| | gaa Glu 210 | | | | | | | | | | | | | | 672 |
| | gac Asp | | | | | | | | | | | | | | 720 |
| _ | ttg Leu | - | | - | | _ | | _ | | | | _ | | | 768 |
| _ | gct Ala | _ | _ | | _ | | _ | _ | _ | | - | | | | 816 |

| | | | | | | _ | | | | | | | | | | | |
|---|-------------|----------------|-------------------|------------|-------------------|------------|------------|-------------------|------------|------------|------------|------------|-------------------|------------|------------|------------|------|
| • | gac Asp | ggc Gly | cag Gln 275 | gcc Ala | cac His | gcc Ala | aaa Lys | gtc Val 280 | Gly ggg | ttc Phe | ttc Phe | caa Gln | agt Ser 285 | cct Pro | tac Tyr | gag Glu | 864 |
| | - | _ | | | gtc Val | _ | | | | _ | _ | | | _ | | _ | 912 |
| | _ | | _ | | gcc Ala | | - | | | | - | | _ | | | | 960 |
| | | _ | | _ | aac Asn 325 | | _ | _ | | | _ | _ | _ | - | _ | | 1008 |
| | | | | | cgt Arg | | | | | | | | tag | | | | 1047 |
| | <212 | L> 32 2> PF | TS | illiı | ım ci | ltrin | num | | | | | | | | | | |
| | <400 Met | | Asn | Gly | Lys 5 | Thr | Phe | Thr | Leu | Ser 10 | Asn | Gly | Val | Lys | Ile 15 | Pro | |
| | Gly | Val | Gly | Phe 20 | Gly | Thr | Phe | Ala | Ser 25 | Glu | Gly | Ser | Lys | Gly 30 | Glu | Thr | |
| | Tyr | Thr | Ala 35 | Val | Thr | Thr | Ala | Leu 40 | Lys | Thr | Gly | Tyr | Arg 45 | His | Leu | Asp | |
| | Cys | Ala 50 | Trp | Tyr | Tyr | Leu | Asn 55 | Glu | Gly | Glu | Val | Gly 60 | Glu | Gly | Ile | Arg | |
| | Asp 65 | Phe | Leu | Lys | Glu | Asn 70 | Pro | Ser | Val | Lys | Arg 75 | Glu | Asp | Ile | Phe | Val 80 | |
| | Cys | Thr | Lys | Val | Trp 85 | Asn | His | Leu | His | Arg 90 | Tyr | Glu | Asp | Val | Leu 95 | Trp | |
| | Ser | Ile | Asp | Asp 100 | Ser | Leu | Lys | Arg | Leu 105 | Gly | Leu | Asp | Tyr | Val 110 | Asp | Met | |
| | Phe | Leu | Val 115 | His | Trp | Pro | Ile | Ala 120 | Ala | Glu | Lys | Asn | Gly 125 | Gln | Gly | Glu | |
| | | | | | | | | | | | | | | | | | |

Pro Lys Ile Gly Pro Asp Gly Lys Tyr Val Ile Leu Lys Asp Leu Thr

Glu Asn Pro Glu Pro Thr Trp Arg Ala Met Glu Lys Ile Tyr Glu Asp

135

145 150 155

| Arg | гуѕ | Ala | Arg | 165 | TIE | GIY | vai | Ser | 170 | пр | 1111 | 116 | Ala | 175 | ьеα | |
|--------------|-------------------------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----|
| Glu | Lys | Met | Ser 180 | Lys | Phe | Ala | Lys | Val 185 | Met | Pro | His | Ala | Asn 190 | Gln | Ile | |
| Glu | Ile | His 195 | Pro | Phe | Leu | Pro | Asn 200 | Glu | Glu | Leu | Val | Gln 205 | Tyr | Cys | Phe | |
| Ser | Lys 210 | Asn | Ile | Met | Pro | Val 215 | Ala | Tyr | Ser | Pro | Leu 220 | Gly | Ser | Gln | Asn | |
| Gln 225 | Val | Pro | Thr | Thr | Gly 230 | Glu | Arg | Val | Ser | Glu 235 | Asn | Lys | Thr | Leu | Asn 240 | |
| Glu | Ile | Ala | Glu | Lys 245 | Gly | Gly | Asn | Thr | Leu 250 | Ala | Gln | Val | Leu | Ile 255 | Ala | |
| Trp | Gly | Leu | Arg 260 | Arg | Gly | Tyr | Val | Val 265 | Leu | Pro | Lys | Ser | Ser 270 | Asn | Pro | |
| Lys | Arg | Ile 275 | Glu | Ser | Asn | Phe | Lys 280 | Ser | Ile | Glu | Leu | Ser 285 | Asp | Ala | Asp | |
| Phe | Glu 290 | Ala | Ile | Asn | Ala | Val 295 | Ala | Lys | Gly | Arg | His 300 | Phe | Arg | Phe | Val | |
| Asn 305 | Met | Lys | Asp | Thr | Phe 310 | Gly | Tyr | Asp | Val | Trp 315 | Pro | Glu | Glu | Thr | Ala 320 | |
| Lys | Asn | Leu | Ser | Ala 325 | | | | | | | | | | | | |
| <213 <213 | 0 > 4 1 > 9' 2 > DI 3 > Pe | AV | illiı | ım c: | itriı | num | | | | | | | | | | |
| | 1 > CI | DS 1) | (978) |) | | | | | | | | | | | | |
| atg | | | | _ | act Thr | | | _ | _ | | | - | _ | | | 48 |
| | | | | | acc Thr | | | | | | | | | | | 96 |
| | | | | | act Thr | | | | | | | | | | | 144 |

| | | | | | | | | | 6 | | | | | | | |
|--------------|-------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|
| tgt · Cys | gcc Ala 50 | | | | _ | | | | | _ | | | | | _ | 192 |
| _ | ttc Phe | _ | _ | | | | - | | _ | _ | | - | | | _ | 240 |
| _ | acc Thr | _ | | | | | | | _ | | | - | - | | | 288 |
| | att Ile | - | - | | _ | _ | _ | | | | - | | - | _ | _ | 336 |
| | ctc Leu | | | | | | | | | | | | | | | 384 |
| | aag Lys 130 | | | | - | | | | _ | | | _ | _ | _ | | 432 |
| | aac Asn | | | | | | _ | _ | _ | | _ | | | | _ | 480 |
| _ | aag Lys | - | | | | | - | | | | | | _ | _ | | 528 |
| | aag Lys | _ | | _ | | _ | _ | _ | _ | | | _ | | _ | | 576 |
| | att Ile | | | | | | | | | | | | | | | 624 |
| | aag Lys 210 | | | | | | | | | | | | | | | 672 |
| | gtt Val | | | | | | | | | | | | | | | 720 |
| | atc Ile | | | | | | | | | | | | | | | 768 |
| | ggt Gly | | | | | | | | | | | | | | | 816 |

| aag Lys . | | | | | | | | | | | | | | | 864 |
|------------------------------|----------------|-------|-------|------|------|------|------|-----|------|------|------|---|---|---|-----|
| ttt Phe | _ | _ | | | _ | - | _ | - | | _ | | | _ | - | 912 |
| aac Asn 305 | _ | _ | _ | | | | | _ | _ | | | | | _ | 960 |
| aag Lys | | | | | tga | | | | - | | | | | | 978 |
| <210 <211 <212 <213 | > 27 > DN | JA | icia: | l Se | quen | ce | | | | | | | | | |
| <220 <223 | | escr: | ipti | on o | f Ar | tifi | cial | Seq | uenc | e: P | rime | r | | | |
| <400 gcca | | cta 1 | tgaag | ggcg | at c | cagt | ac | | | | | | | | 27 |
| <210 <211 <212 <213 | > 29 > Di | NΑ | icia | l Se | quen | ce | | | | | | | | | |
| <220 <223 | | escr | ipti | on o | f Ar | tifi | cial | Seq | uenc | e: P | rime | r | | | |
| <400 cgga | | gtc . | atcg. | aggc | gt g | cagc | tagc | | | | | | | | 29 |
| <210 <211 <212 <213 | .> 2' !> DI | AV | icia | l Se | quen | ce | | | | | | | | | |
| <220 <223 | | escr | ipti | on o | f Ar | tifi | cial | Seq | uenc | e: P | rime | r | | | |
| <400 gcca | | cta | tgtc | taac | gg a | aaga | ct | | | | | | | | 27 |
| <210 <211 <212 <213 | L> 2 2> Di | NΑ | icia | l Se | quen | ce | | | | | | | | | |

```
<220>
· <223> Description of Artificial Sequence: Primer
 <400> 8
 cggatccgtt ataatttcgt agagattca
                                                                    29
 <210> 9
 <211> 21
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Primer
 gatcatcata gcaggagtca t
                                                                    21
 <210> 10
 <211> 21
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Primer
 <400> 10
 gaattcaaca ccagtcagct c
                                                                    21
 <210> 11
 <211> 786
 <212> DNA
 <213> Bacillus megaterium
 <220>
 <221> CDS
 <222> (1)..(786)
 atg tat aaa gat tta gaa gga aaa gta gtt gtc ata aca ggt tca tct
 Met Tyr Lys Asp Leu Glu Gly Lys Val Val Ile Thr Gly Ser Ser
 acc ggt tta gga aaa gca atg gcg att cgt ttt gcg aca gaa aaa gct
                                                                    96
 Thr Gly Leu Gly Lys Ala Met Ala Ile Arg Phe Ala Thr Glu Lys Ala
              20
                                   25
                                                       30
 aaa gta gtt gtg aac tat cgt tcg aaa gaa gaa gct aac agc gtt
                                                                    144
 Lys Val Val Val Asn Tyr Arg Ser Lys Glu Glu Glu Ala Asn Ser Val
          35
 tta gaa gaa att aaa aaa gtg ggc gga gag gct att gcc gtc aaa ggt
                                                                    192
 Leu Glu Glu Ile Lys Lys Val Gly Gly Glu Ala Ile Ala Val Lys Gly
      50
                          55
```

87.

| | • | | | | • | | | | | | | | | | | |
|---|---|---|-------------------|-------------------|-----|---|---|---|---|---|---|---|---|---|---|-----|
| | - | | _ | gag Glu | | - | | | | | _ | | | _ | | 240 |
| | | | | aag Lys 85 | | - | - | | | | | - | | _ | _ | 288 |
| | _ | - | _ | tct Ser | | _ | _ | | | _ | - | | | | _ | 336 |
| | - | - | | tta Leu | _ | | _ | | | | _ | _ | _ | | | 384 |
| | | | | gaa Glu | | _ | | _ | | | - | | | _ | _ | 432 |
| _ | - | | | aaa Lys | | | | | | | _ | | | ~ | _ | 480 |
| _ | | | | atg Met 165 | _ | | _ | | _ | | | | | _ | | 528 |
| | | | | att Ile | _ | - | | | | | | | | | | 576 |
| | _ | | | gct Ala | - | | | _ | _ | | | _ | _ | _ | _ | 624 |
| _ | _ | _ | _ | att Ile | | _ | | | | | | _ | _ | _ | | 672 |
| _ | | _ | _ | gca Ala | | | _ | | | | - | _ | | _ | | 720 |
| | | | | ttt Phe 245 | _ | _ | | | _ | | _ | | | | | 768 |
| | - | | cgc Arg 260 | gga Gly | taa | | | | | | | | | | | 786 |
| | | | | | | | | | | | | | | | | |

<210> 12 <211> 261 <212> PRT <213> Bacillus megaterium

<400> 12

Met Tyr Lys Asp Leu Glu Gly Lys Val Val Val Ile Thr Gly Ser Ser
1 5 10 15

Thr Gly Leu Gly Lys Ala Met Ala Ile Arg Phe Ala Thr Glu Lys Ala
20 25 30

Lys Val Val Asn Tyr Arg Ser Lys Glu Glu Glu Ala Asn Ser Val
35 40 45

Leu Glu Glu Ile Lys Lys Val Gly Glu Ala Ile Ala Val Lys Gly 50 55 60

Asp Val Thr Val Glu Ser Asp Val Ile Asn Leu Val Gln Ser Ala Ile 65 70 75 80

Lys Glu Phe Gly Lys Leu Asp Val Met Ile Asn Asn Ala Gly Met Glu 85 90 95

Asn Pro Val Ser Ser His Glu Met Ser Leu Ser Asp Trp Asn Lys Val

Ile Asp Thr Asn Leu Thr Gly Ala Phe Leu Gly Ser Arg Glu Ala Ile 115 120 125

Lys Tyr Phe Val Glu Asn Asp Ile Lys Gly Thr Val Ile Asn Met Ser 130 135 140

Ser Lys Gly Gly Met Lys Leu Met Thr Glu Thr Leu Ala Leu Glu Tyr 165 170 175

Ala Pro Lys Gly Ile Arg Val Asn Asn Ile Gly Pro Gly Ala Ile Asn 180 185 190

Thr Pro Ile Asn Ala Glu Lys Phe Ala Asp Pro Glu Gln Arg Ala Asp 195 200 205

Val Glu Ser Met Ile Pro Met Gly Tyr Ile Gly Glu Pro Glu Glu Ile 210 215 220

Ala Ala Val Ala Ala Trp Leu Ala Ser Ser Glu Ala Ser Tyr Val Thr 225 230 235 240

Gly Ile Thr Leu Phe Ala Asp Gly Gly Met Thr Gln Tyr Pro Ser Phe 245 250 255

Gln Ala Gly Arg Gly

<210> 13

<211> 27

<212> DNA

<213> Artificial Sequence

| <210> 15 <211> 28 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Primer <400> 15 | <220> <223> Description of Artificial Sequence: <400> 13 gccatggcta tgtataaaga tttagaa | Primer |
|--|--|-----------|
| <223> Description of Artificial Sequence: Primer <400> 14 cggatccgtt atccgcgtcc tgc 23 <210> 15 <211> 28 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Primer <400> 15 | <211> 23 <212> DNA | . · |
| <211> 28 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Primer <400> 15 | <223> Description of Artificial Sequence: <400> 14 | Primer 23 |
| <223> Description of Artificial Sequence: Primer <400> 15 | <211> 28 <212> DNA | |
| | - | Primer |
| | | 28 |